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A Turbulent Channel Flow Apparatus for the Determination of the Adhesion Strength of Microfouling Organisms

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The development of novel, fouling-release surfaces has led to the need for better test methods to evaluate their performance. A water channel has been designed to measure the adhesion strength of microfouling organisms to test surfaces. The apparatus allows six replicate microscope slides to be mounted in a fully-developed, turbulent channel flow. Wall shear stress in the test section can be varied from 0.9–30 Pa over a Reynolds number range of 2,800 to 27,000 based on the bulk mean velocity and channel height. Calibration of the device indicates that the accuracy and repeatability in the wall shear stress is within 4% throughout the range. Experiments using the fouling diatom *Amphora* settled on acid-washed glass slides are presented. The results show significant differences in the shear stress required to remove *Amphora* cells with settlement time. No significant differences among the replicate slides were observed, indicating flow uniformity in the test section.

Keywords: *Amphora*; adhesion strength; flow cell; turbulent channel flow; shear stress

INTRODUCTION

Many researchers have utilized flow cell devices to determine the shear stress necessary to detach bioflims and microfouling organisms from a variety of substrates. Types of flow cell designs have included the radial flow chamber (RFC), laminar channel flow cell, fully-developed turbulent pipe flow and the annular flow cell.

The RFC has been by used in several investigations to assess the relative adhesion strength of microorganisms to a range of test surfaces (*e.g.* Fowler & McKay, 1980; Duddridge *et al.*, 1982; Milne & Callow, 1985; Hyde *et al.*, 1989; Callow *et al.*, 1993). Callow *et al.* (1993) used RFC to study the adhesion of the marine diatom *Amphora coffeaeformis*. The wall shear stresses required to remove 50% of the diatoms from a glass surface and a silane-coupled hydrophobic coating were 5.5 and 12.2 Pa, respectively. It was noted that while the RFC provided a quick method of comparing the adhesion strength of diatoms to different surfaces, the shear stress values obtained with the device should be used with caution. These problems and theoretical aspects relating to the measurement of shear stress in the RFC have

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been discussed at length by Fryer *et al.* (1984; 1985).

Laminar flow cells have also been used in the study of biofilm adhesion strength (for example Pedersen, 1982; Zinn *et al.*, 1999). The arrangement of Pedersen (1982) was designed to allow settlement assays on a number of microscope cover slips and care was given to the hydrodynamic design. Diffusers and a flow stabilizer were incorporated to increase flow uniformity and reduce large-scale vorticity. Flow visualization studies confirmed that the flow in the test cell was quite uniform. Although there are several attractive features to this design its use is limited by the current need to employ microscope slides as templates for test surfaces. The stacked design would present problems with testing coated slides and differences in the spacing between the slides could lead to an order of magnitude difference in the shear stress.

In the flow cell design of Zinn *et al.* (1999) five test coupons were arranged streamwise along a high aspect ratio channel (the ratio of channel width to height). This design allows some development upstream of the coupons, although it is not clear that it is sufficient for fully-developed laminar conditions. Since the slides were arranged side by side, with their long axes perpendicular to the flow, misalignment of a single slide could affect the flow over all the slides downstream of it. This design also provided no means of adjusting the height of individual slides to accommodate coatings of different thickness.

Fully-developed, turbulent pipe flow was used by Picologlou *et al.* (1980) to study the effect of bacterial biofilms on frictional resistance. They found that bacterial biofilms did not significantly alter the wall shear stress over the first 35 h of settlement but subsequently led to a marked increase. The use of a fully-developed pipe flow design allowed the accurate determination of the wall shear stress from a simple measurement of the pressure drop along the pipe. High aspect ratio rectangular channels offer similar advantages and also have well defined flow characteristics (*e.g.* Tiederman *et al.*, 1985; Durst *et al.*, 1998).

The water channel facility used by Tiederman *et al.* (1985) was designed for accurate determination of the wall shear stress and considerable care was given to inlet flow management. The use of a setting chamber with perforated plate diffusers, honeycomb flow straighteners, screens, and large contraction ratio provided the necessary inlet conditions for accurate and repeatable shear stress measurements in the test section. The design also allowed for sufficient test section length to ensure fully-developed conditions.

Annular flow cells have also been used to study the attachment of microbes (Characklis, 1990). These devices consist of concentric cylinders in which the gap is filled with water. The inner cylinder rotates at a rate that is varied based on the shear stress desired. The wall shear stress is determined using the applied torque and the rotation rate. Removable coupons can be mounted on the wall of the outside cylinder to conduct biofilm sampling. The simplicity and the fairly well defined flow pattern generated make this a desirable design. However, the use of standard glass slides in this type of flow cell would be problematic. The coupons would have to be curved and mounted flush if an accurate shear stress determination was to be made.

The main design requirement for the present system was to provide a rapid, repeatable test for determining the shear stress necessary to remove microfouling organisms (algal spores and diatoms) from a range of test surfaces. This necessitated a well characterized flow within a range of velocities and shear stresses appropriate to these organisms. In order to facilitate the application of test coatings and the counting of organisms before and after testing, the apparatus was designed to accommodate several standard microscope slides as replicated test coupons. Based on the literature and these system requirements, a fully-developed, turbulent channel flow was designed, with the following advantages. A fully-developed flow allows accurate

determination of the wall shear stress to which the individual organisms are exposed and the shear stress is nominally constant streamwise. A high aspect ratio (ratio of channel width to height) channel facilitates the mounting of slides and reduces secondary flows that can lead to variation in the shear stress across the channel. Turbulent flows allow higher wall shear stresses to be generated and is more realistic for testing hull coatings since ships operate in the turbulent flow regime (*e.g.* Saunders, 1957; Patel, 1998).

MATERIALS AND METHODS

Water Channel Apparatus

A diagram of the water channel design is shown in Figure 1. The design velocity range in the test section was 0.42–3.3 $m\,s^{-1}$ (Reynolds number based on channel height and bulk mean velocity of 2,800 to 27,000). The resulting wall shear stress could be varied from approximately 0.9–30 Pa. The apparatus accommodated six replicate slides per test.

A 0.56 kW thermoplastic centrifugal pump (McMaster-Carr, Atlanta GA, USA) capable of delivering 2.5 $l\,s^{-1}$ at 9 m of head was used to drive the flow in the water channel. The motor was single phase and operated on 230 V. The flow rate was varied *via* a globe valve on the outlet side of the pump and a ball valve on the recirculation loop. A magnetic flowmeter placed downstream of the pump monitored the flow rate through the system. The flowmeter was an ABB MagMaster model #MFE4ER140311 transmitter with a model #MFE400372801004ER magnetic sensor (ABB Instrumentation, Rochester, NY, USA). The accuracy of the flowmeter was ±0.2% of the reading over the design range of 0.32 to 2.5 $l\,s^{-1}$. This allowed the bulk mean velocity in the test section to be determined to a similar accuracy. A settling chamber (Figure 2) was placed upstream of the test section to improve the flow uniformity, to remove any large-scale vorticity induced by the pump, and to lower the background turbulence intensity in the test section. The walls of the settling chamber were constructed of 13 mm thick cast acrylic sheet. Flow entered the chamber by means of a perforated pipe and then passed through three perforated plates which acted as a diffuser. These were constructed of 20 gauge 304 stainless steel and had open area ratios of 40%, 51%, and 63%, respectively. A polycarbonate honeycomb

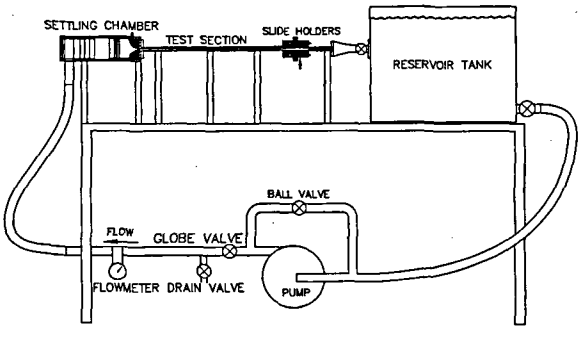

FIGURE 1 Schematic diagram of the water channel design. System components included a centrifugal pump, magnetic flowmeter, settling chamber, test section, digital manometer, discharge tank, and piping. The approximate overall dimensions of the system were 1 m in height, 2.5 m in length, and 1 m in width.

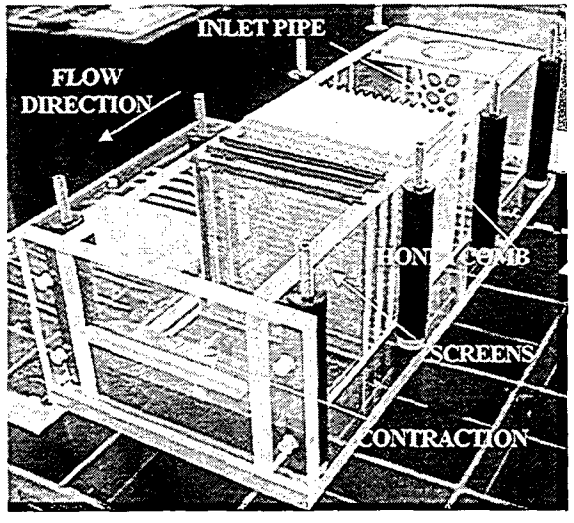

FIGURE 2 Photograph of the settling chamber. The top and perforated plates have been removed to show the inlet pipe, honeycomb, and screens, which acted to break up large-scale vorticity induced by the pump.

(6 mm openings and a length of 75 mm) and a series of three screens (#24 mesh, 60% open area ratio) further reduced large-scale vorticity and freestream turbulence levels. At the end of the settling chamber a two-dimensional nozzle (contraction ratio of 15 : 1) accelerated the flow and reduced the relative magnitude of the background turbulence. Tripping plates, 0.5 mm in height, were placed immediately downstream of the contraction (~ 15% blockage as recommended by Durst *et al.*, 1998). The shear layer trips helped to ensure that the flow in the test section was fully-developed and turbulent at the lower Reynolds number range.

The test section (Figure 3) was 7.19 mm in height (H), 105 mm in width (W), and 1000 mm in length (L) (14.6 : 1 aspect ratio). The section was constructed of cast acrylic to allow optical access for viewing the experiment or for flow measurements with laser-Doppler velocimetry. Four pressure taps were placed from 60H to 100H downstream of the tripping plates. Durst *et al.* (1998) found that 60H is a sufficient length to obtain a fully-developed turbulent channel at Reynolds numbers > 3000. In fully-developed flow the wall shear stress is related to the pressure drop in a high aspect ratio channel by Eqn 1 (Hussain & Reynolds, 1975):

$$\tau_w = -\frac{H}{2}\frac{dp}{dx} \tag{1}$$

where τ_w = wall shear stress, H = channel height, and dp/dx = streamwise pressure gradient.

The mounting apparatus for the six replicate slides was placed 104H downstream of the tripping plates. The slides were placed side by side, with their long axis aligned with the flow. Three were placed on the top of the channel and three on the bottom. Each slide mounting port had an articulating positioning mechanism. This allowed the slide to be positioned flush with the channel wall and variation in coating thickness to be accommodated. The mounting port used a

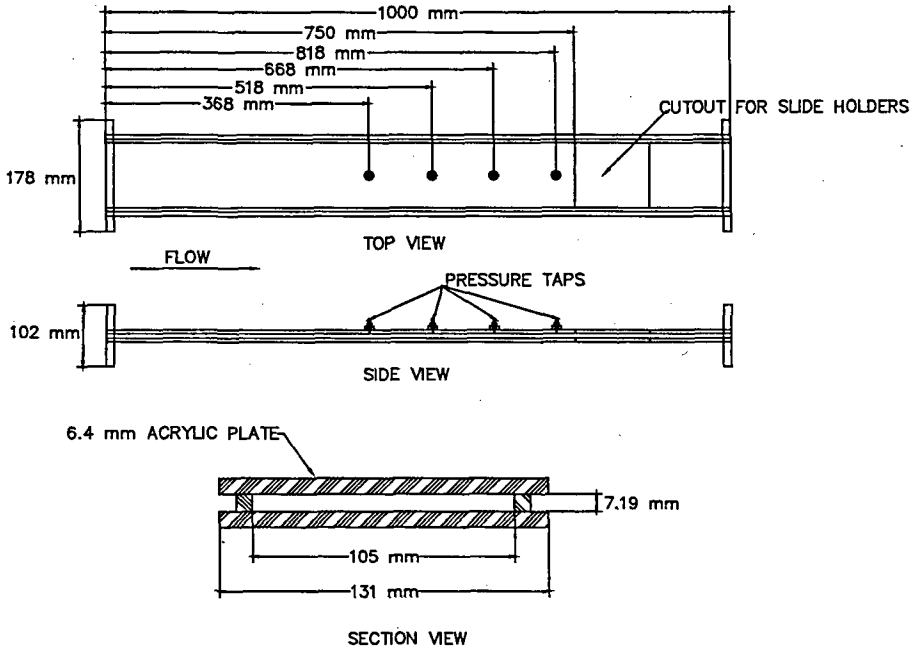

FIGURE 3 Diagram of the test section. The flow moves from left to right and the slides were mounted in slide holders that formed the walls of the channel in the downstream section.

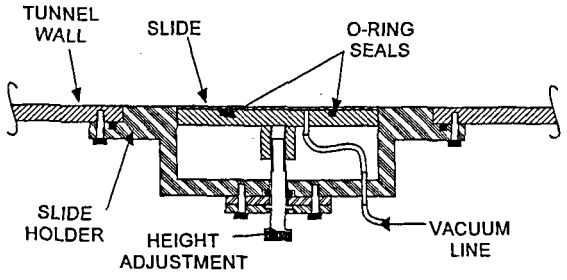

FIGURE 4 Diagram of the slide mounting apparatus. A vacuum applied to o-ring seals on the back side of the slide held it in place. A threaded height adjustment allowed the slide to be positioned flush with the test section wall.

vacuum applied to the reverse side of the slide to hold it in place (see Figure 4).

It should be noted that from a fluid mechanics perspective it would be desirable to have the flow develop entirely over the fouled surface. In that case the effect of the roughness caused by the organisms on the wall shear stress could be evaluated. However, since the organisms tested were small (< 15 μm) and 1000 mm test specimens are unmanageable for a rapid screening test, it was assumed that the wall shear stress was unaltered by the presence of the organisms. This appears to be a satisfactory assumption since the viscous length scale in the channel ranged from 6 to 35 μm over the design velocity range. The roughness Reynolds number, defined as the ratio of the roughness height to the viscous length, therefore remained between about 0.4 and 2.5 in the present experiments. For uniformly distributed sand roughness, a roughness Reynolds number $< \sim 5$ has been shown to cause no increase in the wall shear stress above that of a smooth surface (Schetz, 1993). To ensure the roughness Reynolds number of the test surfaces remains small, the fouling should not be given a significant growth period. Biofilms that were allowed to grow for as little as 96 h have been shown to significantly increase the wall shear stress and alter the turbulence structure in boundary layer flows (Schultz & Swain, 1999).

A digital manometer was used to measure the pressure drop in the test section. The meter was a Validyne differential model #PS309D-1-N-1 (Validyne Engineering, Northridge, CA, USA) with nickel plated stainless steel wetted parts for corrosion resistance. Its range was 0–150 mm H_2O with an accuracy of ±0.25% of full scale. The use of several pressure tap spacings allowed the manometer to operate in the upper part of its range and gave more accurate pressure measurements. The wall shear stress in the test section could be determined to within ±4% over the entire velocity range using this arrangement.

The flow from the test section exited into a diffuser pipe and into a discharge tank. The tank had a large volume (265 l), which increased the time necessary for all the fluid to make a complete circuit of the water channel. This reduced the build-up of heat in the seawater. Its relatively large size also allowed flow exiting the test section to settle before being recirculated.

Amphora Detachment Assay

In order to evaluate the applicability of the flow cell to analyses of adhesion strength in microfouling organisms, experiments were conducted using the diatom *Amphora coffeaeformis* var. *purpusilla* which is commonly used in experimental fouling studies (Cooksey, 1981; Woods & Fletcher, 1991; Callow *et al.*, 1993). Cultures of *Amphora* were grown in Guillard's F2 medium (Guillard & Ryther, 1962) on an illuminated orbital incubator at 20°C. Log phase cells were allowed to settle under gravity then washed twice in Instant Ocean seawater (Aquarium Systems) in order to prevent carry over of nutrients, thereby preventing cell division during the course of the experiment. This washed suspension of cells was placed on a magnetic stirrer to break up cell clumps and then filtered through 35 μm nylon mesh to produce a suspension of mostly single cells. Settlement was carried out using acid-washed glass microscope slides (2 h in 50% methanol : 50% concentrated hydrochloric acid followed by 2 h in 100% concentrated hydrochloric acid). Slides were placed in individual compartments of

polystyrene culture dishes (In Vitro Systems & Services, GmbH) and 10 ml of *Amphora* culture, previously diluted in Instant Ocean to give an absorbance of 0.06 at 664 nm were added. The cells were allowed to settle onto the glass slides under gravity for 2 h and then washed gently by passing each slide back and forth 10 times in a beaker of Instant Ocean to remove unattached cells. Slides were placed in clean dishes containing Instant Ocean inside an illuminated incubator at 16°C for varying periods of time.

Two experimental procedures were carried out with the settled cells. The first studied the strength of adhesion with increasing contact time on the glass slides after the standard 2 h settlement period. Cells adhered to the slides were challenged at the highest flow rate of 2.3 l s^{-1} for 5 min (wall shear stress of 28.2 Pa; Reynolds number based on the bulk mean velocity and channel height of ~21,000). In the second experiment the strength of adhesion was examined after 5 h total contact time (*i.e.* including the 2 h initial settlement period), using a range of flow rates, each of 5 min duration. In both experiments the mean of 6 replicate control slides, not exposed to flow, was used to calculate the percentage removal of cells at each slide position in the flow cell, at each time point or flow rate. The mean level of settlement on control slides was 373 cells $mm^{-2} \pm 56$ (SE).

RESULTS AND DISCUSSION

Shear Stress Calibration Measurements

Calibration of the water channel consisted of a series of pressure gradient measurements along the test section. These calibration tests were carried out over the entire flow rate range. Freshwater was used as the working fluid. The temperature of the water ranged between 24°–25°C during the tests. Six, standard uncoated microscope slides were mounted in the test section. Care was taken to bleed all the air from the manometer tubing and to zero the instrument prior to the measurements. The wall shear stress

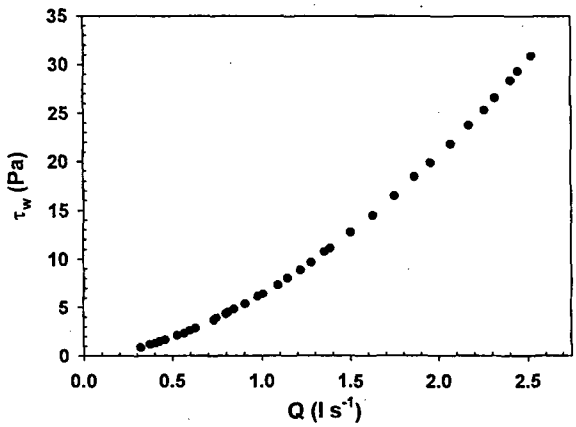

FIGURE 5 Calibration results of wall shear stress *vs* flow rate for the water channel apparatus.

in the test section was calculated using Eqn 1. The wall shear stress vs flow rate results are presented in Figure 5. The 95% confidence limits on the wall shear stress are $< \pm 4\%$ over the entire flow rate range.

In order to generalize the results of the calibration, the wall shear stress and the velocity in the test section were normalized to the Fanning friction factor and the Reynolds number using Eqns 2 and 3, respectively:

$$f = \frac{4\tau_w}{(1/2)\rho \bar{U}^2} \tag{2}$$

where f = Fanning friction factor, ρ = fluid density, and $\bar{U}$ = bulk mean velocity in the test section.

$$\mathrm{Re} = \frac{H\bar{U}}{\nu} \tag{3}$$

where H = channel height, Re = Reynolds number, and ν = kinematic viscosity of the fluid.

The normalized results are presented in Figure 6, together with the results of Dean (1978) for turbulent channel flow. The present individual data points all agree to within ±4% with Dean's formula, and the regression agrees to within ±2.5% with the individual readings over the range of Reynolds numbers tested.

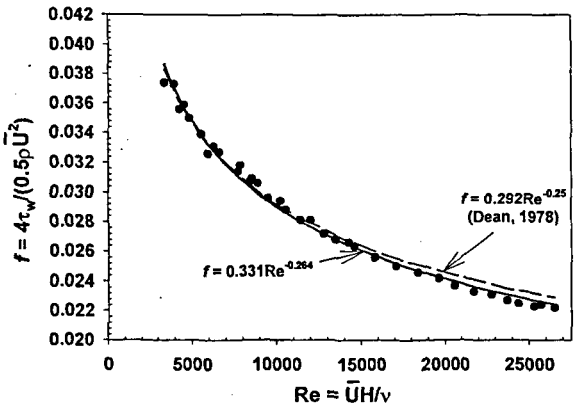

FIGURE 6 Non-dimensionalized calibration results of friction factor *vs* Reynolds number for the water channel apparatus. The solid line is the least squares power law fit of the present results. The dashed line shows the results of Dean (1978) for turbulent channel flow.

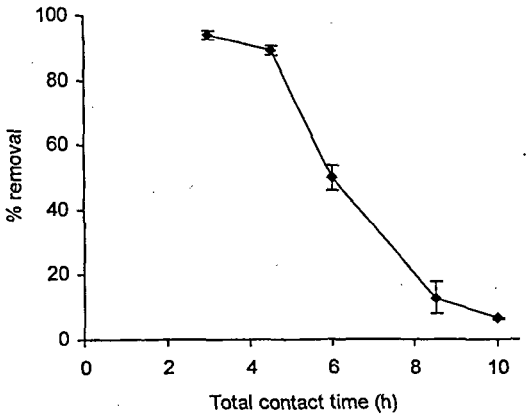

FIGURE 7 The effect of increasing post-settlement time under static conditions ('contact time') on the strength of adhesion of *Amphora* cells. Cells were settled on glass slides for 2 h, unattached cells were removed then slides were aged for various time periods before each was exposed to 5 min flow at 2.3 $l s^{-1}$ (wall shear stress = 28.2 Pa). Percentage removal data were determined from the mean of 6 replicate slides compared with control slides unexposed to shear stress. Bars = ±the SE of the mean derived from arcsine transformed data.

The power law formula in Figure 6 allows the channel operator to generate a predetermined wall shear stress by monitoring the temperature of the seawater and adjusting only the system flow rate, thus eliminating the more tedious pressure gradient measurements used in the calibration process.

Amphora Adhesion Assays

In the first experiment, the relationship between contact time and strength of adhesion was sigmoidal (Figure 7). The percentage of cells removed under a standard flow regime decreased greatly between 4 and 8 h, indicating an increasing strength of attachment with increasing post-settlement time under static conditions. The detachment of cells was approximately 50% after 6 h at the employed flow rate (*viz.* 2.3 $l s^{-1}$). One way analysis of variance showed significant differences between the numbers of cells present over the time course ($F_{5,30} = 27.54$, $p < 0.05$). Two way analysis of variance showed that there were no significant differences between the slide positions ($F_{5,270} = 1.63$, $p > 0.05$) indicating flow uniformity in the test section.

Amphora is a raphid diatom which produces extracellular polymeric substances (EPS) for the dual purposes of adhesion and locomotion (Edgar & Pickett-Heaps, 1984; Hoagland *et al.*, 1993; Wetherbee *et al.*, 1998). During settlement under gravity the sedimentation of cells through the water column proceeds at a relatively rapid rate, but the final stages leading to contact adhesion between the cell EPS and the glass surface take longer. Furthermore, initial adhesion is reversible as the cells adjust their position by gliding locomotion (Edgar & Pickett-Heaps, 1984; Wetherbee *et al.*, 1998) which may account for the low adhesion strength before 4 h. The increasing strength of adhesion after 4 h may be due to processes associated with secondary or permanent adhesion (Wetherbee *et al.*, 1998) including the secretion of additional components of EPS *via* the raphes, along with reorganisation and secondary modifications to the secreted EPS such as cross-linking by divalent cations (Cooksey, 1981; Cooksey & Wigglesworth-Cooksey, 1995) or phenolics (Wustman *et al.*, 1997).

In the second experiment, sensitivity of cells to a range of wall shear stresses was measured by exposing cells with a total contact time on the

glass slides under static conditions of 5 h (including the 2 h settlement period) to a range of flow rates (this contact time was chosen since at the highest flow rate approximately 80% of cells were removed (Figure 7)). Approximately 40% of the settled cells were dislodged at the lowest flow rate of 0.4 $l s^{-1}$ (wall shear stress of ~ 1.4 Pa) (Figure 8). These weakly attached cells may represent that proportion of the cell population still engaged in initial reversible attachment (Edgar & Pickett-Heaps, 1984). Alternatively, some cells may have reaggregated within the original suspension, as small clumps bonded through relatively weak cohesive forces compared with the stronger adhesive forces bonding cells to the surface.

Increased flow rate above 0.9 $l s^{-1}$ (5.7 Pa) significantly increased the detachment of cells in a linear fashion (ANOVA $F_{5,30} = 55.9$ $p < 0.05$), which probably reflects variation in adhesion strength within the cell population. Approximately 10% of cells remained attached at the highest flow rate used (2.3 $l s^{-1}$, 28.2 Pa). The wall shear stress necessary to remove 50% of cells with a total contact time on the slides of 5 h, was approximately 5 Pa. This value compares with a critical wall shear stress value of 5.5 Pa previously obtained in radial flow cell studies of this species settled on glass for 2 h (Callow *et al.*, 1993).

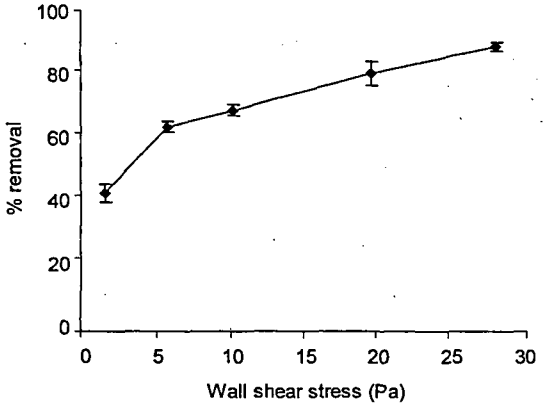

FIGURE 8 The effect of flow rate expressed as wall shear stress (Pa) on the adhesion of *Amphora* cells. Cells settled for 5 h were challenged with a range of flows and the percentage removed determined by comparison with control slides not exposed to shear stress. Each point is derived from 6 replicate slides. Bars = ±the SE of the mean derived from arcsine transformed data.

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